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Author(s)	Ko, Jae-Hong; Asano, Atsushi; Kon, Yasuhiro; Watanabe, Tomomasa; Agui, Takashi
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FULL PAPER

Characterization of the Chicken PKR : Polymorphism of the gene and Antiviral Activity against Vesicular Stomatitis Virus

Jae-Hong Ko,[1] Atsushi Asano,[1] Yasuhiro Kon,[2] Tomomasa Watanabe,[3] and Takashi Agui[1]*

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Abstract

The double-stranded RNA-dependent protein kinase (PKR) is induced in mouse and human cells on treatment with interferon. In this study, we have cloned and determined the nucleotide sequences of chicken PKR cDNA in various chicken breeds. Chicken PKR was a 550-amino-acid protein as deduced from the cDNA open reading frame (ORF), and there were specific domains (two double-stranded RNA binding domains (DRBDs) and numerous kinase subdomains) characterized in RNA binding proteins and kinase families. Furthermore, it was suggested that chicken PKR was polymorphic. Transfected cell clones expressing chicken PKR mRNA were demonstrated to confer antiviral responses to vesicular stomatitis virus, except for Koshamo type-3 (KS-3). KS-3 PKR, which has an amino acid substitution at position 507 (Arg to Gln), showed amphibious antiviral responses. This specific amino acid substitution was considered to determine the antiviral function of chicken PKR in addition to essential domains as DRBDs and kinase subdomains.

Key Words : Chicken ; PKR ; Polymorphism ; Vesicular stomatitis virus

Introduction

Interferons (IFNs) are a family of regulatory cytokines that possess a wide range of biological activities[27]. Type I IFNs are produced in virtually all cell types after viral infection, and then induce translation of a set of genes that mediate an antiviral state. A double-stranded RNA (dsRNA) - dependent protein kinase, designated PKR[28,30], is one of

[1]Laboratory of Experimental Animal Science, Department of Disease Control and[2] Laboratory of Anatomy, Department of Biomedical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan
[3]Animal Breeding and Reproduction, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan
* Corresponding author : Takashi Agui, Laboratory of Experimental Animal Science, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan
Tel/Fax : +81-11-706-5106, E-MAIL : agui@vetmed.hokudai.ac.jp

those proteins. PKR plays a central role in the regulation of protein synthesis in virus-infected and IFN-treated cells[5,29,30] and has been implicated in the regulation of other activities including cell proliferation and tumor suppression[20].

Human PKR is a 551-amino-acid protein with a molecular mass of about 62 kDa as deduced from the cDNA open reading frame (ORF)[17,23,30,37]. By contrast, the mouse and rat PKR proteins are somewhat smaller, 515[13,36] and 514 amino acids[22], respectively. The N-terminal region of the PKR protein possesses a repeated motif of double-stranded RNA binding domain (DRBD), which contains about 20 amino acid residues[8,10,15,21,26,33]. In addition, there are eleven catalytic subdomains of protein serine/threonine kinase are in the C-terminal half of PKR[30,31].

Activation of PKR enzymic activity coincides with autophosphorylation at serine and threonine residues[9,18]. Activated PKR catalyzes the phosphorylation of protein synthesis eukaryotic initiation factor 2 (eIF-2) at serine 51 of the α subunit. This modification of eIF-2 leads to the formation of a stable complex between eIF-2 and the guanine nucleotide exchange factor eIF-2B, resulting in the inhibition of translation[6,12]. On the other hand, a number of viruses including influenza virus, adenovirus, Epstein-Barr virus, vaccinia virus, herpes simplex virus type I and hepatitis C virus have specific mechanisms to defeat PKR[2,14]. Nevertheless, it is obvious that PKR plays a key role in the protection at the early stage of the infection of these viruses.

Despite its important role in inhibition of viral replication, only few studies were carried out about PKR of domestic animals. Here, we have isolated and sequenced a full-length cDNA encoding PKR from chicken. Furthermore, we analyzed the antiviral activity of chicken PKR to vesicular stomatitis virus (VSV) with transfected BALB3T3cells. Our present study revealed that chicken PKR has an inhibitory function to replication of VSV.

Materials and Methods

Chicken Breeds

Seven Japanese native chicken breeds, Kojidori (KJ), Koshamo (KS), Nagoya (NG), Rhode Island Red (RI), Hokkaido Shamo (SHK), Satsumadori (SM), and White Leghorn (WLK) were used in this study.

Cell Culture

Embryonic fibroblasts were established from 11-day-old chicken embryos. The fibroblasts were incubated in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Trace Biosciences, NSW, Australia). Secondly passaged cells in culture were used for experiments. A murine fibroblast cell line, BALB 3T3, was maintained in the same culture medium.

Cloning and Sequencing of Chicken PKR

RNA prepared from embryonic fibroblasts treated with the IFN inducer, poly (I)/(C) for 7h[16,32] was used for synthesis of double-stranded cDNA as a PCR template. The 5' and 3' rapid amplification of cDNA ends (RACE) of chicken PKR cDNA was performed using the PCR primers NE5-F and NE2-R, respectively (Table1), which were designed based on the sequence showing homology between the known other mammalian PKRs, and the adaptor primers AP1 (5'-CCA TCC TAA TAC GAC TCA CTA TAG GGC-3') and AP2 (5'-ACT CAC TAT AGG GCT CGA GCG GC-3'), using a Marathon cDNA amplification kit (Clontech, Palo Alto, CA, USA). NE2-F and NE3-R were generated based on the sequences of progressively amplified 5'-and 3'-RACE to obtain the ORF of chicken PKR

Table 1. PCR Primers for Characterization of Chicken PKR

Name	Position	Sequence
	nt	
NE 1 -F	234-253	5'-ACTTGAATGAGAAATCGGAG-3'
NE 2 -F	393-412	5'-CTCAGATATTGCATCGAGAT-3'
NE 3 -F	510-529	5'-CCTGAGTTTGAAGTTGTGGT-3'
NE 4 -F	691-710	5'-TGCTCCCTCCAGTTGACTAC-3'
NE 1 -R	1009-990	5'-ATACCCCATGACTCAGACTG-3'
NE 5 -F	1413-1432	5'-TATTATTGCAGCTGGGAAGG-3'
NE 2 -R	1501-1482	5'-TGGATGAAGAGGCAAGAAAC-3'
NE 6 -F	1820-1839	5'-GCTGGGATTGATTTGGTTTG-3'
NE 3 -R	2111-2092	5'-ATCACATGCCACAAGACAGC-3'
NE 4 -R	2754-2735	5'-CAAATGCCACAGGAATGAGC-3'
NE 5 -R	2943-2924	5'-CGTTAAAATCAGATGGCTAT-3'

cDNA. Amplified ORF fragments were cloned into pGEM-T Easy vector (Promega, Madison, WI, USA) and DNA sequences were determined using an ABI PRISM Big Dye terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA, USA) with an ABI PRISM 377 genetic analyzer (Applied Biosystems).

Construction of Chicken PKR Expression Vector and Transfection into 3T3 Cells

The complete coding region of chicken PKR cDNA was cloned into the *Not I* site of the expression vector pCI-neo (Promega), which contains the human cytomegalovirus immediate-early enhancer/promoter and the neomycin phosphotransferase gene. After chicken PKR cDNA constructed with the expression vector was transfected into BALB 3T3 cells using the FuGENE 6 transfection reagent (Roche Diagnostics, Indianapolis, IN, USA), essentially as recommended by the manufacturer, transfected clones were selected in medium containing 500 μg/ml of G 418 (Sigma, Saint Louis, MO, USA).

RT-PCR

Total RNA (2 μg) from 3T3 cells was reverse-transcribed using oligo dT primer and 50 U of reverse transcriptase (ReverTra Ace, Toyobo, Tokyo, Japan) in a total volume of 10 μl. NE3-F and NE1-R were used as primers for the PCR of the expressed chicken PKR gene in transfected 3T3 cells. As an internal control for the estimation of approximate degree of the chicken PKR gene expression, mouse β-actin mRNA was used. PCR was performed with 1 μl of first-strand cDNA as described above, using 2.5 U of Taq polymerase (Sigma) in 1.5 mM $MgCl_2$, 0.2 μM of each primer, and 20 μM of each dNTP in a total volume of 25 μl, as recommended by the supplier. The cycling profile comprised an initial denaturation step for5min at 94℃ followed by 35 cycles at 94℃ for1min, 58℃ for1min, 72℃ for 1min, and a final extension at 72℃ for5min. An aliquot of each PCR product was electrophoresed on a 1.5% agarose gel and visualized with ethidium bromide.

VSV Infection Experiment

Recombinant VSV (VSVΔ G*-G) carrying the green fluorescent protein (GFP) gene instead of the G protein gene was kindly provided by Dr. M.A. Whitt (University of Tennessee, Memphis, Tenn.) through Dr. A. Takada (Institute of Medical Science, University of Tokyo). Infectivity of VSVΔ G*-G in 3T3cell clones was determined by counting the number of GFP-expressing cells in 10-20 microscopic fields as shown in previous paper[16,35]. At least three independent experiments were carried out for each clone.

Statistical Analysis

Data were expressed as means ± standard errors of the means. Statistical significance was evaluated by one-factor analysis of variance with Fisher's protected least significant difference test. $P < 0.05$ were considered statistically significant.

Results

Cloning of cDNA Encoding Chicken PKR

Oligonucleotide primers NE5-F and NE2-R have been synthesized based on regions of homology between the known other mammalian PKRs (Table 1). We treated fibroblasts from chicken embryos with poly(I)/(C) to induce expression of PKR mRNA. Substantially, strong PKR mRNA expression was found in the culture with poly(I)/(C) (data not shown). Additional primers, shown in Table 1, were designed based on the sequences of amplified 5'-and3'-RACE products and used to amplify cDNA fragments for cloning and sequencing of chicken PKR. Sequence analysis showed that chicken PKR was a 550-amino-acid protein as deduced from the cDNA ORF, and there were specific domains characterized in kinase families such as two DRBDs and numerous kinase subdomains (Fig. 1). Homologies of the chicken PKR in the amino acid sequence with other species PKR were 34.9, 32.7, and 33.6% for human, mouse, and rat, respectively.

Primary Structure of the Chicken PKR Protein

Comparisons of regulatory domains of the PKR between chicken and other species were shown in Table 2. The consensus motifs of DRDB were highly conserved in chicken PKR. On the other hand, the conserved features of protein kinases have been reported by the alignment of the catalytic domain amino acid sequences from 65 different members of the protein kinase family[11]. In the human PKR nine subdomains with conserved kinase motifs show striking homology[23]. Therefore, we compared nine kinase motifs of chicken PKR with those of other animals, except for subdomains V and X, since kinase motief is not conserved in these two subdomains (Table 3). The kinase motifs of chicken PKR showed high homology with those of other animals, 74% for human, 82% for mouse, and 77% for rat. These comparisons of kinase motifs revealed that chicken PKR possess well-conserved kinase domains, and homologies in the C-terminal half containing protein kinase domain between chicken and human, mouse, and rat were 47, 48, and 47%, respectively. Furthermore, we analyzed the nucleotide sequences of chicken PKR cDNA from one or two embryos each of many breeds, and compared them with each other. Five independent combinations on the basis of the amino acid substitutions at three residues were observed in the chicken PKR cDNA examined (Table 4).

Chicken PKR of Some Breeds Confers Antiviral Activity to VSV

To confirm the biological function of chicken PKR, we established cell lines constitutively expressing chicken PKR mRNA. SHK-1, RI, KS-2 and KS-3, WLK-1, and KJ-2 were used as representatives of the group as classified in Table 4. Their PKR cDNA constructs with plasmid pCI-neo were transfected into a murine fibroblast cell line, BALB 3T3, which is derived from the BALB/c mouse that has been reported as a sensitive strain to VSV infection[7]. After transfection, we selected the clones constitutively expressing respective PKR mRNA using G418. Then we analyzed the antiviral property of each clones against VSVΔG*-G. As shown in Figure 2, the cell lines expressing chicken PKR from RI, KS-2, WLK-1, and KJ-2 showed antiviral responses to VSV infection, with a lower number of infected cells than those of control 3T3 cells, except for one clone (f3) of KJ-2 that showed no amplification PKR fragment in RT-PCR. However, the cell lines expressing chicken PKR from KS-3 conferred amphibious response. Thus, two clones (d1 and d2) showed antiviral responses, while one clone (d3) did not. Therefore, further independent infection experiments using other clones from KS-3 were performed. Three of six cell clones

```
   1                CATCGTGCGCGTCATAGAGTCTGACAGCCTCTCGCCTGAGAGTGCAGGCT
  51  GGCGCGCCTCGCTTCTGCTCTGAAGGAAGCAGTTCTCATTCCTTATGGAGAAACGTTACT
 111  GATTTCTCTCAGCATCCACTGAGTGATTCAGCCTCTTCCAGAAATCTGCTGAGAAATCAC
 171  CCTTCATCACTAAACTCGCCGTTCTGCTTTCTGAACCTGTAATCTCAAATTGCAAACCAA
 231  ACGACTTGAATGAGAAATCGGAGTTAATTCTCTGTGACCGTTCTCCTCCTGGCACCGTTT
 291  TGCCCTGTCTCAGCAGTGCTCTGGCAGAACGGGGGCTGTCTCTGTAGCACTGCGCGAGGC
 351  TCAGGCACGGGCCTGAATATTTCTCTGCAGAATCTTTTGGTACTCAGATATTGCATCGAG

 411  ATGGACCGAGAGTGCATGGCCAAGATCAACAGGTACTGCCAGAAGAATAAATACAAGCTC
   1   M  D  R  E  C  M  A  K  I  N  R  Y  C  Q  K  N  K  Y  K  L

 471  GATTACGTCGACGTGGACATGAGAGGCCCATCTCACGACCCTGAGTTTGAAGTTGTGGTT
  21   D  Y  V  D  V  D  M  R  G  P  S  H  D  P  E  F  E  V  V  V

 531  AAAATAAATGGTGTGAAGTATGGTACAGGCGTTGGTAAGAGTAAGAAGGAAGCAAAAGCT
  41   K  I  N  G  V  K  Y  G  T  G  V  G  K  S  K  K  E  A  K  A

 591  GCAGCAGCAGCAAAAACGTGGGACATGATTGAGCCAAAGCAAGATAATCTTTTGAATGTG
  61   A  A  A  A  K  T  W  D  M  I  E  P  K  Q  D  N  L  L  N  V

 651  CATACTTCGGATGTAGTGATATCTCCTCTACCTGCGGATGTGCTCCCTCCAGTTGACTAC
  81   H  T  S  D  V  V  I  S  P  L  P  A  D  V  L  P  P  V  D  Y

 711  ATCAGCCTGCTAAATAAGTACTCACAAAAGACATTGCAGCTAGTTGATTACAATAACATA
 101   I  S  L  L  N  K  Y  S  Q  K  T  L  Q  L  V  D  Y  N  N  I

 771  AACCGTACCGGAGACCCCCACGCTCCAATGTATTCTTGTAGCTGTGTAATTAGTGGTCAT
 121   N  R  T  G  D  P  H  A  P  M  Y  S  C  S  C  V  I  S  G  H

 831  GTATATGGAAATGGGAAGGGAAACTCTCTAGCTGTTGCAAAACAAGCCGCTGCAAAAGAA
 141   V  Y  G  N  G  K  G  N  S  L  A  V  A  K  Q  A  A  A  K  E

 891  GCATATGAAGTGCTACAGAAGCAGGAATCTTTCAGACTCAAAAGTGAAAACTCCAGTAAT
 161   A  Y  E  V  L  Q  K  Q  E  S  F  R  L  K  S  E  N  S  S  N

 951  ACAGTTCATGAACTCAGTAATTCCTCTGGAGGGCAGAGTCAGTCTGAGTCATGGGGTATT
 181   T  V  H  E  L  S  N  S  S  G  G  Q  S  Q  S  E  S  W  G  I

1011  ACCTTTAAAGACTCAGCTACAGGCTTGACAGAAGAAGTGAAAGACATGTCCCTATCTGAA
 201   T  F  K  D  S  A  T  G  L  T  E  E  V  K  D  M  S  L  S  E

1071  GAGCCTTCAAATTGTCGGAGATATGTGCCAAGTTCTGCCATAAAAGCCAGAAGAGTATTG
 221   E  P  S  N  C  R  R  Y  V  P  S  S  A  I  K  A  R  R  V  L

1131  GCACCTAATTTTGATAATGCAAGAAATGAGCAGAAAAAAGCCATGTCAAATATCTGCAGA
 241   A  P  N  F  D  N  A  R  N  E  Q  K  K  A  M  S  N  I  C  R

1191  AGTAAGAGTGAGGCAAATGATGAGTATACTGTGGATGAAAGGTTTCGTCAGCAGTATAAA
 261   S  K  S  E  A  N  D  E  Y  T  V  D  E  R  F  R  Q  Q  Y  K

1251  AATATAGAGCCTATTGGTAAAGGTGGCTTTGGGAATGTCTTTAAAGCAACATCAAGGACT
 281  N  I  E  P  I  G  K  G  G  F  G  N  V  F  K  A  T  S  R  T

1311  GATGAGAGAACCTATGCAATCAAACGAGTTGAGTTAATAAATAGGAACGTAAAGCGTGAA
 301   D  E  R  T  Y  A  I  K  R  V  E  L  I  N  R  N  V  K  R  E
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Fig. 1. Nucleotide and deduced amino acid sequences of chicken PKR cDNA. Numbers to the left of the sequence show the positions of nucleotides and amino acids. The double-stranded RNA binding motifs are underlined. The conserved subdomains of the protein kinases are shown in the boxes.

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1371  GTGAAAGAGCTCGCGAATCTTGAACATGAAAACATAGTGCGATATTATTGCAGCTGGGAA
 321   V  K  E  L  A  N  L  E  H  E  N  I  V  R  Y  Y  C  S  W  E

1431  GGAACTGACCATATGATTTACCCAGACTCAAGTAAGAATTCTATCGTAGCTGTTTCTTGC
 341   G  T  D  H  M  I  Y  P  D  S  S  K  N  S  I  V  A  V  S  C

1491  CTCTTCATCCAAATGGAATTATGTGAACAAGGGCCATTGGAAAAATGGATTGAAAATAAT
 361   L  F  I  Q  M  E  L  C  E  Q  G  P  L  E  K  W  I  E  N  N

1551  GGAGGAAACCCAAACTATCATATGATGGCACAAGACAAATTTTTACAAATACTGAAAGGA
 381   G  G  N  P  N  Y  H  M  M  A  Q  D  K  F  L  Q  I  L  K  G

1611  GTGGAGTATATTCATTCTAAAGATTTAATTCACAGAGACCTCAAGCCTCAGAATATATTC
 401   V  E  Y  I  H  S  K  D  L  I  H  R  D  L  K  P  Q  N  I  F

1671  CTATCATATGAAGGTAAAATAAAAATTGGTGACTTTGGTCTCGTGACTTCTGTGACATAC
 421   L  S  Y  E  G  K  I  K  I  G  D  F  G  L  V  T  S  V  T  Y

1731  AACCCTCTGACTAAGAACCGAGGAACACAGTCATATATGGCACCAGAACAGTTTGGGGAC
 441   N  P  L  T  K  N  R  G  T  Q  S  Y  M  A  P  E  Q  F  G  D

1791  AGATATGGAAAGGAAGTAGACATTTATGCGCTGGGATTGATTTGGTTTGAAATTCTTTCT
 461   R  Y  G  K  E  V  D  I  Y  A  L  G  L  I  W  F  E  I  L  S

1851  GCATTGGTCAGTCATCATGAGAAAAACAAGGTATGGCAGGATGTTAGAGGAGGTGACCTT
 481   A  L  V  S  H  H  E  K  N  K  V  W  Q  D  V  R  G  G  D  L

1911  CCACTGAACTTCACCAAGCGATTTAAAATCCAGGTACCCATAATCAAAAAGATGCTTTCA
 501   P  L  N  F  T  K  R  F  K  I  Q  V  P  I  I  K  K  M  L  S

1971  GAAGACCCCTCAAAAAGATGCTCTGCATCTCAAATAATTGATATTTTGAAGTCTCGTGAT
 521   E  D  P  S  K  R  C  S  A  S  Q  I  I  D  I  L  K  S  R  D

2031  AAAGACAACTCGCATAAAGCTTACAGTCACTAAATGCCATTTTAGAAGGCTTTCAGACTG
 541   K  D  N  S  H  K  A  Y  S  H  *

2091  TGCTGTCTTGTGGCATGTGATTCAGACCTTTCTGAATTGTTCAGTCATGTTTATATTAAT
2151  TACAAGATTTCTCCCTTTTAAGACCAGAGGAGTTCATGTGCTTGACTGGAAAGGCTACTT
2211  TTGGCAGAAGGAATACTTAGATACACACATGGTTTATTCTTGACTGAATTTCACTTCTTT
2271  TAGGTGAACTTACTTTTTAAAATCAGGTCTTCCTTAAGTATTTAGCTTTTGGGAAAGATA
2331  TTTGTTCTGTACTTTTGAAAGCCTCAGTTTGGTTTTTTTGTTTTGTTCTTCCTTATAGGA
2391  ACAGGTAGGGTCCTCTTAATACAGAAATGCTCTCCAAGTTTTCTCAATAGCAACCATTTA
2451  AAAACCTTTCTCAAGAGAGCACCTCAGACATTCTCTCCTATACAGTACGGTTTTAATCGC
2511  CTATTATGGCATTGAGGCACATTTCACAGATTATAGGTGTACCTGAACACCTCTGCTGGC
2571  CTTACTGTCAGCTCAACATAAGGTATGAACTGATTGACCGTTACTCTTTGGTTATATACT
2631  TAAGAGATTTCTCGTTTGACTAAATAAGAACAACTGTGCAGGGAAGAAAAGATGGGCAAA
2691  TTATTCCTTCTGCCTCTCTTTTTTGAATAGTTTGAAAAGGTGCAGCTCATTCCTGTGGCA
2751  TTTGCTTAATTCCATAAAGGAAAATGCCCTAAGACTTTTAAGCAGGAAAGTATGATATGC
2811  ATTAGAGGTAAGGTAGCATATGACTTTTTTCAAGTGTTAAACAGATTAAAGGTGGGTGGC
2871  TAAAATATGAATGAGTATTTGCATTTTTATAGTCCTGTGGAAATTGTTGTTAAATAGCCA
2931  TCTGATTTTAACGTTTTTTTGTATGAATTCAAGTTGTCCCCCCTCCCCTTATAATAAATG
2991  TTATTTTTCCGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
```

Table 2. Comparison of Regulatory Domain (RD) in Various Animal PKRs

	RD	
	DRBD 1	DRBD 2
Chicken PKR	GVGKSKKEAKAAAAAKTWDMIE	GKGNSLAVAKQAAAKEAY-EVL
Human PKR	GEGRSKKEAKNAAA-KLAVEIL	GTGSTKQEAKQLAAKLAYLQIL
Mouse PKR	AKGRSKQEARNAAA-KLAVDIL	GSGVTKQEAKQLAAKEAY-QKL
Rat PKR	GEGRSKQEAKNNAA-KLAVEIL	GFGANKKEAKQLAAKNAY-QKL
Consensus	GXGRSKXEAKNAAA-KLAVXIL	GXGXXKXEAKQLAAKXAY-QXL
	A K RAN A TWD E	L V A LE

DRBD ; dsRNA binding domain.

Table 3. Comparison of Kinase Domains (KD) in PKRs of Various Species

	KD								
	I	II	III	IV	VI	VII	VIII	IX	XI
Chicken	IGKGGFGNV	AIKRV	E	NIV	GVEYIHSKDLIHRDLKPQNI	IGDFGL	GTQSYMAPE	DIYALGL	IKKMLSEDPSKR
PKR	IGSGGFGQV	VIKRV	E	NIV	GVDYIHSKKLIHRDLKPSNI	IGDFGL	GTLRYMSPE	DLYALGL	LQKLLSKKPEDR
Human PKR	IGLGGFGQV	AIKRV	E	NIV	GVEYIHSKGLIHRDLKPGNI	IGDFGL	GTLQYMSPE	DIFALGL	LKKLLSEKPKDR
Mouse PKR	IGSGGFGQV	AIKRI	E	NIV	GVDYIHSKGLIHRDLKPGNI	IGDFGL	GTPQYMSPE	DIFALGL	LQKLLSSKPRER
Rat PKR									
	IGXGGFGQV	AIKRV	E	NIV	GVXYIHSKXLIHRDLKPXNI	IGDFGL	GTXXYMSPE	DIXALGL	LXKLLSXKPXXR
Consensus	N	V I					A	L	I M D

Table 4. Amino Acid Substitutions in PKR from Various Chicken Breeds

Nucleotide (amino acid) / Breeds	967 (186)	1110 (234)	1930 (507)
KJ-1, KS-1, SHK-1, and SM-1	G (Ser)	A (Ile)	G (Arg)
KS-2, NG, RI, SHK-2, and SM-2	T (Ile)	A (Ile)	G (Arg)
KS-3	T (Ile)	A (Ile)	A (Gln)
WLK-1	G (Ser)	G (Val)	G (Arg)
KJ-2 and WLK-2	T (Ile)	G (Val)	G (Arg)

KJ, Kojidori ; KS, Koshamo ; NG, Nagoya ; RI, Rhode Island Red ; SM, Satsumadori ; SHK, Hokkaido Shamo ; WLK, Hokkaido White Leghorn.

expressing KS-3 showed antiviral responses and the other three did not. In addition, no nucleotide changes were detected in sequence analysis of RT-PCR fragments from 3T3 cell lines transfected with KS-3 (data not shown).

Discussion

PKR is a serine/threonine protein kinase, whose activity is regulated by double-stranded RNA. It is normally present in low amounts in mammalian cells. However, its expression is induced by INFs in response to viral infection[30]. Analysis of chicken PKR, in this study, showed that chicken PKR was a 550-amino-acid protein as deduced from the cDNA ORF. Multiple copies of the DRBD are found in many of the RNA binding proteins including PKR of human and mouse, and only one copy is found in other related proteins including NS34-Rot, E3L-Vv, PAC1-Yac, and RNaseIII-Ec[30]. It was confirmed that DRBD was conserved in chicken PKR as in that of human, mouse, and rat (Table2). In addition, the conserved features of protein kinases have been reported by the alignment of the catalytic domain amino acid sequences from 65 different members of the protein kinase family[11]. Analysis of the human PKR reveals

striking homologies in the nine subdomains with conserved kinase motifs[23]. In Table 3, chicken PKR showed a high similarity in kinase motifs to PKRs from other animals. Our results of sequence analysis in this study revealed that PKR is conserved very well among various chicken breeds.

The replication of encephalomyocarditis virus[24], HIV-1[4,25], vaccinia virus[19], and VSV[34] is reduced in cell culture by overexpression of the cDNA encoding wild-type PKR but not by expression of the mutant PKR with amino acid substitution in the catalytic subdomain II (K296R), which lacks kinase activity. In the result of sequence analysis, PKRs from various chicken breeds showed no amino acid change in this region, suggesting that chicken PKR has normal kinase activity. In mutant $PKR^{0/0}$ mice with the disruption in the catalytic domain, the antiviral responses to influenza virus and vaccinia virus are normal[1]. However, these mutant mice are predisposed to lethal intranasal infection by VSV[3], and embryonic fibroblasts derived from these mice treated with recombinant IFN-α display a reduced antiviral state against VSV compared to those from wild-type parental mice[31]. In our result of infection experiment with VSV, 3T3 cell lines transfected with chicken PKR cDNA were resistant to VSV except for KS-3, which showed amphibious response (Fig. 2). By the sequence analysis of the RT-PCR products from the KS-3 clones, any mutation was not detected. A possible explanation for the

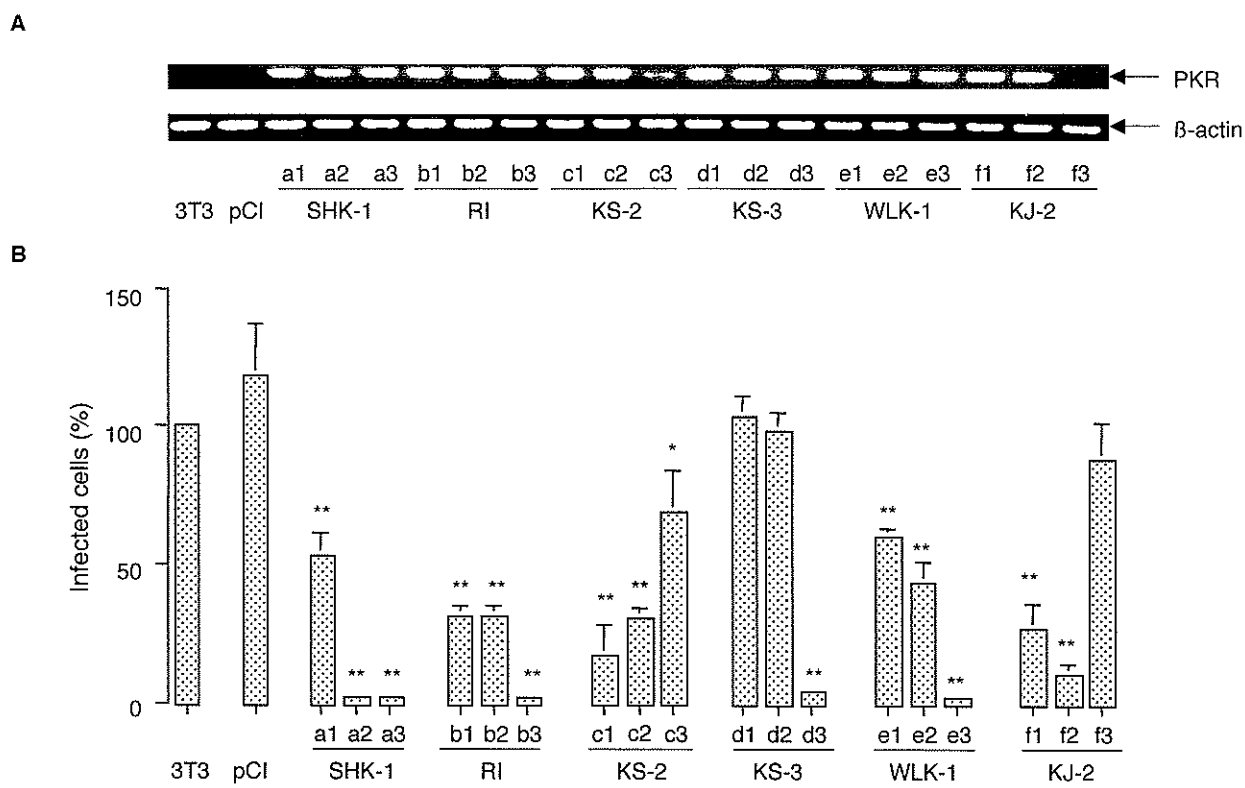

Fig. 2. Infectivity of 3T3 cells transfected chicken PKRs from various breeds against VSVΔG*-G. (A) The expression of PKR mRNA. Total RNA from transfected 3T3 cells was used for RT-PCR. Top panel, PKR; Bottom panel, β-actin. 3T3, parental3T3cells; pCI, 3T3 cells transfected pcI-neo vector only. (B) The infectivity of VSVΔG*-G in PKR cDNA-transfected cell lines. The infectivity on parental 3T3 cells is expressed as 100%. The values are expressed as means ± standard errors of the means (n = 10). $P < 0.05$ (*) or $P < 0.01$ (**) compared with 3T3 cells are indicated.

amphibious result is that PKR from KS-3possesses less antiviral activity than other breed PKRs and exerts an antiviral response only when a large amount of the PKR protein exists in the transfected cells. It is likely that efficiency of translation varies in each clone, even though the transcription occurs at the same level. In the present status, however, it is impossible to clarify this speculation, since anti-chicken PKR is not available. KS-3had Gln, instead of Arg in other chicken PKR, at position 507 (Table 4). Comparison of PKR sequence with other animals revealed that amino acid 507 (Arg or Gln) of chicken PKR was not in either kinase subdomain nor DRBD. These results suggest that specific region of C-terminus containing amino acid 507 is also responsible for antiviral function in chicken PKR. In order to confirm this hypothesis it may be necessary to determine how this substitution influences the antiviral activity of chicken PKR using artificial mutant PKR.

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